

We Claim:

1. A composition comprising a plurality of beads, wherein each of said plurality of beads comprises a plurality of bound polynucleotides, wherein the polynucleotides in the composition are heterogeneous, and wherein on at least 1 % of said beads the plurality of bound polynucleotides is homogeneous.
2. The composition of claim 1 wherein on at least 5 % of said beads the plurality of bound polynucleotides is homogeneous.
3. The composition of claim 1 wherein on at least 10 % of said beads the plurality of bound polynucleotides is homogeneous.
4. The composition of claim 1 wherein on at least 50 % of said beads the plurality of bound polynucleotides is homogeneous.
5. The composition of claim 1 wherein the plurality of bound polynucleotides is greater than 100.
6. The composition of claim 1 which is a liquid.
7. The composition of claim 6 which comprises agarose.
8. The composition of claim 1 wherein the polynucleotides in the composition differ by a single nucleotide polymorphism (SNP).
9. The composition of claim 1 wherein the polynucleotides in the composition differ in the presence or absence of a mutation.
10. The composition of claim 1 wherein the polynucleotides in the composition differ in the presence or absence of an insertion.
11. The composition of claim 1 wherein the polynucleotides in the composition differ by the presence or absence of a polymorphism.
12. The composition of claim 1 wherein the beads are magnetic.
13. The composition of claim 1 wherein at least one species of polynucleotide is labeled with a fluorescent dye.
14. The composition of claim 13 wherein the labeling is via a labeled oligonucleotide.
15. The composition of claim 13 wherein the labeling is via one or more labeled antibodies.

16. The composition of claim 1 wherein the bound polynucleotides were made by amplification of a template in a test sample, wherein the beads on which the plurality of bound polynucleotides is homogeneous comprise at least a first and a second species of polynucleotide, wherein the beads comprising the first species of polynucleotide and the beads comprising the second species of polynucleotide are present in the composition in the same ratio as the first and second species of polynucleotide were present in the test sample.
17. A liquid composition comprising a plurality of microemulsions forming aqueous compartments wherein at least a portion of said aqueous compartments comprise:
  - a bead;
  - a polynucleotide template; and
  - oligonucleotide primers for amplifying said template;wherein at least a portion of the oligonucleotide primers is bound to the bead.
18. The liquid composition of claim 17 which comprises forward and reverse oligonucleotide primers.
19. The liquid composition of claim 17 wherein said aqueous compartments have an average diameter of 0.5 to 50 microns.
20. The liquid composition of claim 17 wherein at least one in 10,000 of said aqueous compartments comprise a bead.
21. The liquid composition of claim 17 wherein from 1/100 to 1 of said aqueous compartments comprise a bead.
22. The liquid composition of claim 17 wherein from 1/50 to 1 of said aqueous compartments comprise a polynucleotide template molecule.
23. The liquid composition of claim 17 wherein the bead is magnetic.
24. The liquid composition of claim 17 wherein the average number of template molecules per aqueous compartment is less than 1.
25. The liquid composition of claim 17 further comprising a DNA polymerase and deoxyribonucleotides.
26. The liquid composition of claim 17 wherein the average diameter of said aqueous compartments is from 1 to 10 microns, inclusive.

27. The liquid composition of claim 17 wherein the average diameter of said aqueous compartments is from 11 to 100 microns, inclusive.
28. The liquid composition of claim 17 wherein the average diameter of said aqueous compartments is about 5 microns.
29. The liquid composition of claim 17 wherein each oligonucleotide primer is at least 12 nucleotides in length.
30. The liquid composition of claim 17 wherein each oligonucleotide primer is from 25 to 55 nucleotides.
31. The liquid composition of claim 17 wherein binding of said oligonucleotide primers to said bead is covalent.
32. The liquid composition of claim 17 wherein binding of said oligonucleotide primers to said bead is via a biotin-streptavidin binding pair.
33. The liquid composition of claim 32 wherein said forward or reverse oligonucleotide primers that are bound to said bead comprise at least two biotin moieties.
34. The liquid composition of claim 17 wherein the aqueous compartments comprise agarose.
35. A method for analyzing nucleotide sequence variations, comprising:
  - forming microemulsions comprising one or more species of analyte DNA molecules;
  - amplifying analyte DNA molecules in the microemulsions in the presence of reagent beads, wherein the reagent beads are bound to a plurality of molecules of a primer for amplifying the analyte DNA molecules, whereby product beads are formed which are bound to a plurality of copies of one species of analyte DNA molecule;
  - separating the product beads from analyte DNA molecules which are not bound to product beads;
  - determining a sequence feature of the one species of analyte DNA molecule which is bound to the product beads.

36. The method of claim 35 further comprising the step of isolating product beads which are bound to a plurality of copies of a first species of analyte DNA molecule from product beads which are bound to a plurality of copies of a second species of analyte DNA molecule.
37. The method of claim 36 wherein the step of isolating is performed using fluorescence activated cell sorting.
38. The method of claim 36 further comprising the step of recovering the first species of analyte DNA molecule from the product beads.
39. The method of claim 36 further comprising the step of amplifying the first species of analyte DNA molecule from the isolated product beads.
40. The method of claim 38 further comprising the step of determining the sequence of the first species of analyte DNA molecule.
41. The method of claim 35 wherein the step of amplifying converts less than 10 % of the reagent beads present in the microemulsions into product beads.
42. The method of claim 35 wherein prior to the step of separating, the microemulsions are broken by addition of one or more detergents.
43. The method of claim 35 wherein the step of determining is performed by hybridization to oligonucleotide probes which are differentially labeled.
44. The method of claim 35 wherein the relative or absolute amounts of product beads comprising one or more sequence features is determined.
45. The method of claim 44 wherein the relative or absolute amounts are determined using flow cytometry.
46. The method of claim 35 wherein the step of amplifying employs additional copies of the primer which are not bound to the reagent bead.
47. The method of claim 35 wherein the analyte DNA molecules are genomic DNA.
48. The method of claim 35 wherein the analyte DNA molecules are cDNA.
49. The method of claim 35 wherein the analyte DNA molecules are PCR products made from genomic DNA.
50. The method of claim 35 wherein the analyte DNA molecules are PCR products made from cDNA.

51. The method of claim 35 wherein the analyte DNA molecules are derived from a single individual.
52. The method of claim 35 wherein the analyte DNA molecules are derived from a population of individuals.
53. The method of claim 35 wherein the reagent beads are magnetic.
54. The method of claim 35 wherein the step of determining a sequence feature is performed by extension of a primer with one or more labeled deoxyribonucleotides.
55. A probe for use in hybridization to a polynucleotide that is bound to a solid support, comprising:

an oligonucleotide with a stem-loop structure having a photoluminescent dye at one of the 5' or 3' ends, wherein said oligonucleotide does not comprise a quenching agent at the opposite 5' or 3' end.

56. The probe of claim 55 which hybridizes to a wild-type selected genetic sequence better than to a mutant selected genetic sequence.
57. The probe of claim 55 which hybridizes to a mutant genetic sequence better than to a wild-type genetic sequence.
58. A pair of molecular probes comprising:

a first oligonucleotide with a stem-loop structure having a first photoluminescent dye at one of the 5' or 3' ends, wherein said first oligonucleotide does not comprise a quenching agent at the opposite 5' or 3' end, wherein said first oligonucleotide hybridizes to a wild-type selected genetic sequence better than to a mutant selected genetic sequence; and

a second oligonucleotide with a stem-loop structure having a second photoluminescent dye at one of the 5' or 3' ends, wherein said second oligonucleotide does not comprise a quenching agent at the opposite 5' or 3' end, wherein said second oligonucleotide hybridizes to the mutant selected genetic sequence better than to the wild-type selected genetic sequence; wherein the first and the second photoluminescent dyes are distinct.

59. A method for isolating nucleotide sequence variants, comprising:

forming microemulsions comprising one or more species of analyte DNA molecules;

amplifying analyte DNA molecules in the microemulsions in the presence of reagent beads, wherein the reagent beads are bound to a plurality of molecules of a primer for amplifying the analyte DNA molecules, whereby product beads are formed which are bound to a plurality of copies of one species of analyte DNA molecule;

separating the product beads from analyte DNA molecules which are not bound to product beads;

isolating product beads which are bound to a plurality of copies of a first species of analyte DNA molecule from product beads which are bound to a plurality of copies of a second species of analyte DNA molecule.

60. The method of claim 59 wherein the step of isolating is performed using fluorescence activated cell sorting.
61. The method of claim 59 further comprising the step of recovering the first species of analyte DNA molecule from the product beads.
62. The method of claim 59 further comprising the step of amplifying the first species of analyte DNA molecule from the isolated product beads.
63. The method of claim 59 further comprising the step of determining the sequence of the first species of analyte DNA molecule.